

CLAIMSWhat is claimed is:

1. A method for cloning a non-human mammal through a nuclear transfer process
 - 5 comprising:
 - (i) obtaining desired differentiated mammalian cells to be used as a source of donor nuclei;
 - (ii) obtaining at least one oocyte from a mammal of the same species as the cells which are the source of donor nuclei;
 - 10 (iii) enucleating said at least one oocyte;
 - (iv) transferring the desired differentiated cell or cell nucleus into the enucleated oocyte;
 - (v) simultaneously fusing and activating the cell couplet to form a first transgenic embryo;
 - 15 (vi) culturing said first transgenic embryo(es) until it reaches at least the 2-cell developmental stage; and
 - (vii) using at least one of the cells of said first transgenic embryo as a donor cell for the production of a transgenic animal through at least a second round of nuclear transfer so as to produce a second transgenic embryo; and
- 20 2. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from mesoderm.
3. The method of claim 1, wherein said donor differentiated mammalian cell to be used
 - 25 as a source of donor nuclei or donor cell nucleus is from endoderm.
4. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from ectoderm.
- 30 5. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from fetal somatic tissue.
6. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from fetal somatic cells.

7. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from a fibroblast.
- 5 8. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an ungulate.
9. The method of either claims 1 or 8, wherein said donor cell or donor cell nucleus is from an ungulate selected from the group consisting of bovine, ovine, porcine, equine, caprine and buffalo.
- 10 10. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an adult non-human mammalian somatic cell.
- 15 11. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is selected from the group consisting of epithelial cells, neural cells, epidermal cells, keratinocytes, hematopoietic cells, melanocytes, chondrocytes, B-lymphocytes, T-lymphocytes, erythrocytes, macrophages, monocytes, fibroblasts, and muscle cells.
- 20 12. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an organ selected from the group consisting of skin, lung, pancreas, liver, stomach, intestine, heart, reproductive organ, bladder, kidney and urethra.
- 25 13. The method of claim 1, wherein said at least one oocyte is matured *in vivo* prior to enucleation.
- 30 14. The method of claim 1, wherein said at least one oocyte is matured *in vitro* prior to enucleation.
15. The method of claim 1, wherein said non-human mammal is a rodent.

16. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is a non-quiescent somatic cell or a nucleus isolated from said non-quiescent somatic cell.
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17. The method of either claims 1 or 8, wherein the fetus develops into an offspring.
18. The method of claim 1, wherein said at least one oocyte is enucleated about 10 to 60 hours after initiation of *in vitro* maturation.
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19. The method of claim 1, wherein a desired gene is inserted, removed or modified in said differentiated mammalian cell or cell nucleus prior to insertion of said differentiated mammalian cell or cell nucleus into said enucleated oocyte.
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20. The resultant offspring of the methods of claims 1 or 19.
21. The resultant offspring of claim 19 further comprising wherein the offspring created as a result of said nuclear transfer procedure is chimeric.
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22. The method of claim 1, wherein cytocholasin-B is used in the cloning protocol.
23. The method of claim 1, wherein cytocholasin-B is not used in the cloning protocol.
24. The method of claim 1, wherein said differentiated mammalian cells to be used as a source of donor nuclei is from an *in vitro* matured oocyte.
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25. The method of claim 24 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at most at the 40-cell stage of development.
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26. The method of claim 24 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at the 32-cell stage of development.

27. The method of claim 24 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at the 16-cell stage of development.
- 5 28. The method of claim 24 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at the 8-cell stage of development.
29. The method of claim 1, wherein said differentiated mammalian cells to be used as a
10 source of donor nuclei is from an in vivo matured oocyte.
30. The method of claim 29 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at most at the 40-cell stage of development.
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31. The method of claim 29 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at the 32-cell stage of development.
- 20 32. The method of claim 29 wherein the cells of said first transgenic embryo are utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at the 16-cell stage of development.
33. The method of claim 29 wherein the cells of said first transgenic embryo are
25 utilized for the generation of at least one said second transgenic embryo when said first transgenic embryo is at the 8-cell stage of development.
34. The method of claim 1, further comprising transferring said second transgenic embryo into a host mammal such that the embryo develops into a fetus.
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35. The resultant offspring of the methods of claims 24 or 29.